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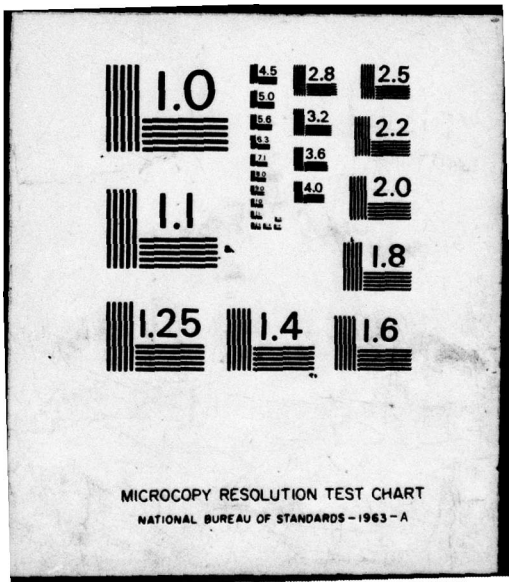
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A STUDY OF THE EFFECT OF ELF ELECTROMAGNETIC FIELDS UPON DROSOPHILA MELANOGASTER

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FINAL REPORT, NOVEMBER 1976

FOREWORD

This study was performed under subcontract to IIT Research Institute for the U.S. Naval Electronic Systems Command under Contract No. N00039-71-C-0111, IITRI-E6185.

All aspects of ELF electromagnetic field treatment of the animals were performed at the IIT Research Institute laboratories in Chicago. Additionally, IIT Research Institute scientists coded both treated and control cultures which were subsequently tested at the University of Notre Dame. Results of all experimentation were recorded for the various coded categories and provided to IIT Research Institute for decoding.

Respectfully submitted,

Harvey A. Bender

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SUMMARY

Drosophila melanogaster were exposed to 20 volts per meter rms and 2 gauss rms at CW frequencies of 45 and 75 Hz for a period of 48 hours. The classical Muller-5 technique for the detection of sex-linked lethals was employed to study mutation rate. No indication of mutagenic effect was found.

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1. INTRODUCTION

This *Drosophila* (fruit fly) study was performed in 1971 as part of a program to determine possible biologic effects of ELF electromagnetic environments similar to those envisaged in the vicinity of the Navy's proposed ELF Communication System, e.g., Projects Sanguine and Seafarer. The study was designed to serve as a confirmation study of an earlier fruit fly study[1/] performed by another laboratory. The earlier study reported unusual and unexpected effects of a 48 hour exposure of *Drosophila melanogaster* to extremely low frequency (ELF) electromagnetic fields. Consequently, this investigation was patterned as closely as possible to the procedures detailed in the report of the outside investigation.

2. EXPERIMENTAL DESIGN

The classical Muller-5 technique for the detection of sex-linked lethals was employed. Although more sensitive techniques now currently are available, the Muller-5 technique was employed to parallel the study of Coate et al which utilized this methodology. Treatment of the animals was carried out by the IIT Research Institute in Chicago. Subsequent to treatment, experimental and control animals were transported to the *Drosophila* Research Laboratory of the University of Notre Dame, where the animals were reared, appropriate crosses undertaken, and data compiled.

2.1. Description of Muller-5 Test

The method of detecting sex linked lethals utilizes the Muller-5 stock which classically has been used to demonstrate the relationship between X-irradiation dosage and mutation rate.

The Muller-5 stock $In(1)sc^{S1L}sc^{8R}+S$, sc^{S1} sc^{8} w^{a} B contains two inversions of the X chromosome the long *scute*^{8} inversion which extends from about 0.1 to about 65 on the X chromosome map and a smaller inversion

within which extends from about 17 to 38. These two inversions markedly reduce crossing over along the entire length of the X chromosome. The stock also carries the recessive mutants scute^{8}, a scute^{s1}, and apricot eye, and the dominant marker Bar eye. The effect of the scute genes in the homozygous females and the hemizygous males is to add bristles particularly on the mesonotum. Apricot shows because it is homozygous and Bar eye behaves as a dominant. Heterozygous females are recognized by their Bar eyes; the other traits are not phenotypically expressed. All traits manifest in the hemizygous Muller-5 males. Both males have good viability and fertility. The Muller-5, or Base stock, has been used routinely to detect sex-linked recessive mutations.

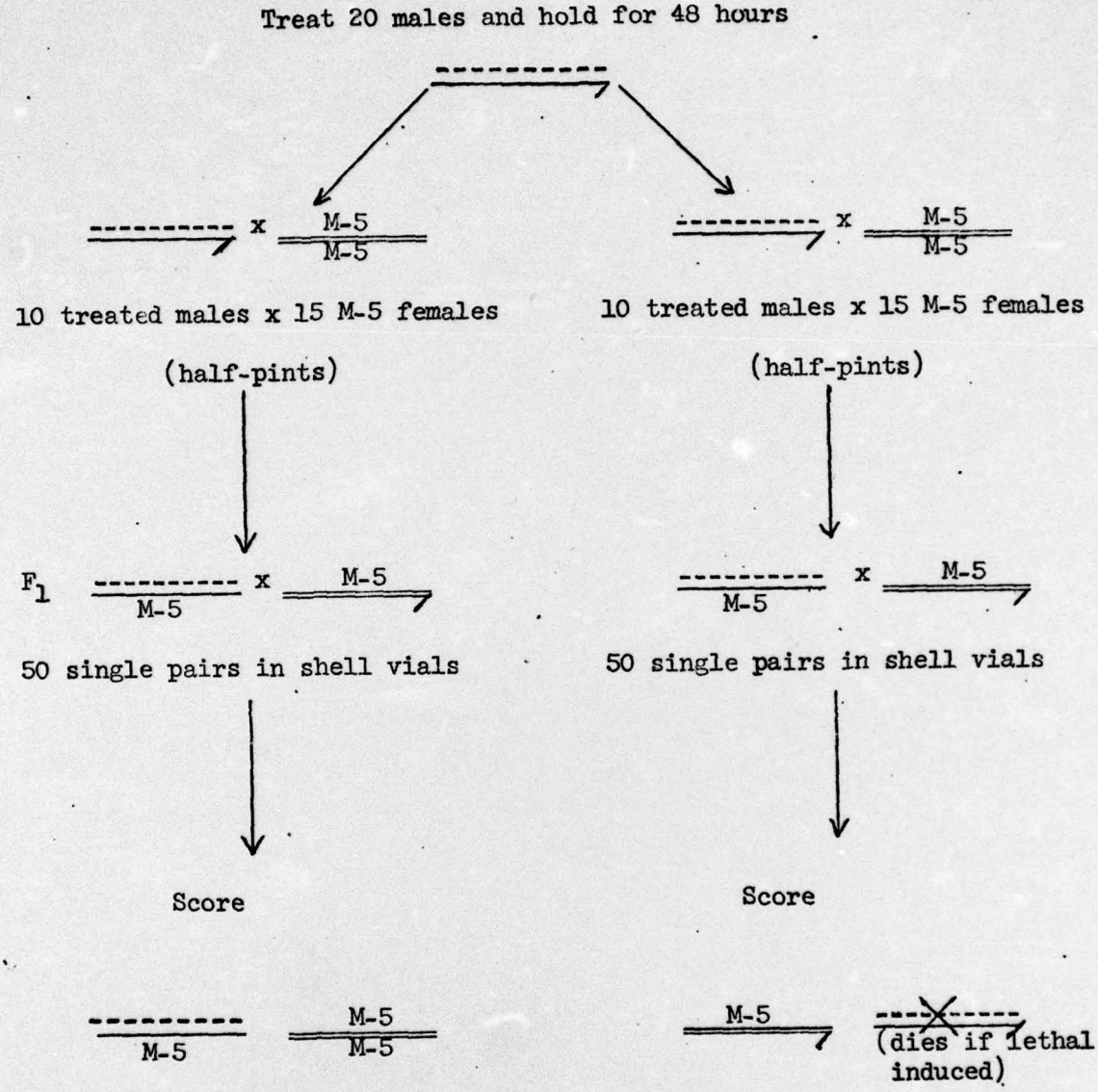
If experimental treatment induces a dominant lethal the effect is immediate. Either the dominant lethal kills the egg or sperm in which it occurs or if the gametes are able to function the resulting zygote can not survive. A recessive lethal can be carried in the heterozygous condition with little effect. It kills the individual only when homozygous or hemizygous.

2.2. Culture Methods and Conditions

Two stocks of Drosophila melanogaster were used in this study: wild-type Oregon-R and an inversion containing, multiple mutant stock In(1)sc^{S1L}sc^{8R}+S, sc^{S1} sc^{8} w^{a} B (the so-called Basc or Muller-5 stock). These stocks have been maintained in culture in the Department of Biology, University of Notre Dame, Indiana. The original stock was obtained from the Drosophila stock center, California Institute of Technology. Animals were cultured in half pint milk bottles or 1,000cc shell vials containing a "standard" Drosophila medium consisting of yellow cornmeal, unsulfured molasses, brewers yeast, agar, and the mold inhibitor propionic acid. Cultures were maintained in a constant temperature room at 25±1°C and 75±5% relative humidity.

In this study, treated males were crossed to Muller-5 females and F_1 heterozygous females were then crossed to Muller-5 F_1 males. Two types of females should appear in the F_2 generation-homozygous Muller-5 females and heterozygous females carrying treated chromosomes. If no lethal is induced on the treated chromosome, wild-type males will appear along with Muller-5 males. If no wild-type males appear, a lethal mutation on the X chromosome is indicated. A reduction in the number of wild males would indicate the possible presence of a semi-lethal. In order to maintain the lethal mutation for further study the stock may be continued by selecting heterozygous females each generation. It would not be necessary to select virgins since their brothers are always Muller-5 males.

The procedure used in this study is diagrammed in Figure 1 which is slightly modified from the outside study. Twenty adult Oregon-R males per each of three replicates were etherized lightly and transferred to treatment containers. The containers were treated for 48 hours. Twenty flies per each of three replicates served as controls. Following treatment, the flies were held for 48 hours and then each replicate of twenty males was divided into two groups of ten males and placed into half pint milk containers containing 15 virgin Muller-5 females. After appearance of larvae, the parents were removed from the culture bottles. After F_1 flies emerged, 50 single pair matings were set in shell vials from each of the half pint cultures utilizing F_1 heterozygous females and F_1 Muller-5 males. As soon as F_2 larvae appeared, the parents were removed from the culture containers. As F_2 flies emerged over an eight day period they were categorized according to sex and male phenotype. Each F_2 vial was considered to represent an original F_1 X chromosome and fly counts were totaled by vials.



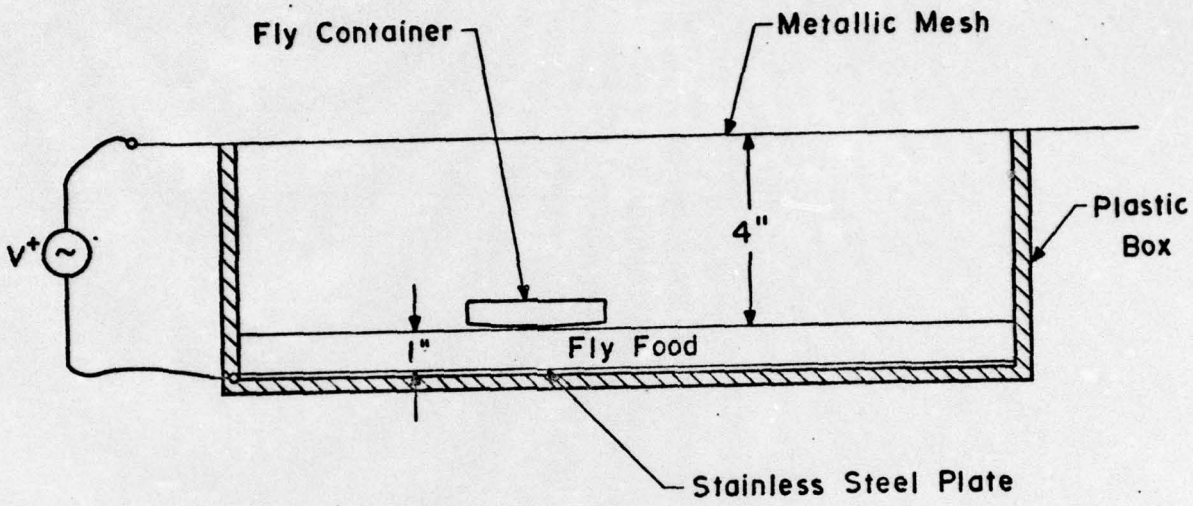
1. above set in triplicate for 45 Hz, 75 Hz, and control
2. total of 300 chromosomes for each series

Figure 1. Procedure used in fruit fly study

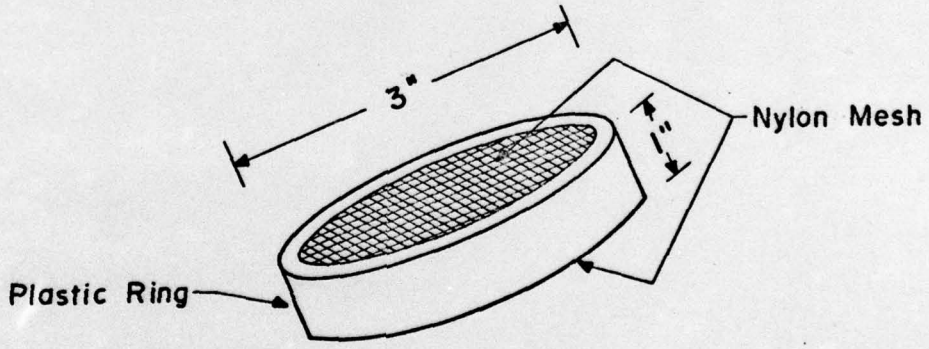
2.3. Electromagnetic Field

The treatment for this study was ELF electromagnetic fields at 45 Hz and 75 Hz. An electric field level of 20 volts per meter rms and a magnetic field level of 2 gauss rms were applied. One group of fruit flies was exposed to the 45 Hz fields and a second group was simultaneously exposed to the 75 Hz fields in the same room. A third group was maintained during this time in the same room as a control group and was not exposed to either field.

A sketch of the apparatus used to provide a uniform electric field is presented in Figure 2. The electric field is established between two parallel planes. One is a stainless steel plate placed in the bottom of a 17x15x5 inch plastic box. The other is a metallic mesh placed on top of the box. To ensure that the electric field is not distorted by the required food, the entire surface of the stainless steel plate was uniformly covered with food. Since the food is highly conductive compared to air, the applied voltage appears between the top of the food and the metallic mesh and a uniform electric field is established in that space. Because the length and width of the metallic planes are large compared to their spacing, a uniform field is established over the central volume where the fruit flies were placed. In this region the electric field intensity is the ratio of the plate voltage to plate spacing. The flies are contained by plastic rings covered on top and bottom with nylon mesh. These rings are placed on top of the food thereby making the food accessible through the mesh. A photograph of three fly containers in place on the food is presented in Figure 3.



E-FIELD APPARATUS



FLY CONTAINER

Figure 2 ELECTRIC FIELD APPARATUS AND FLY CONTAINER

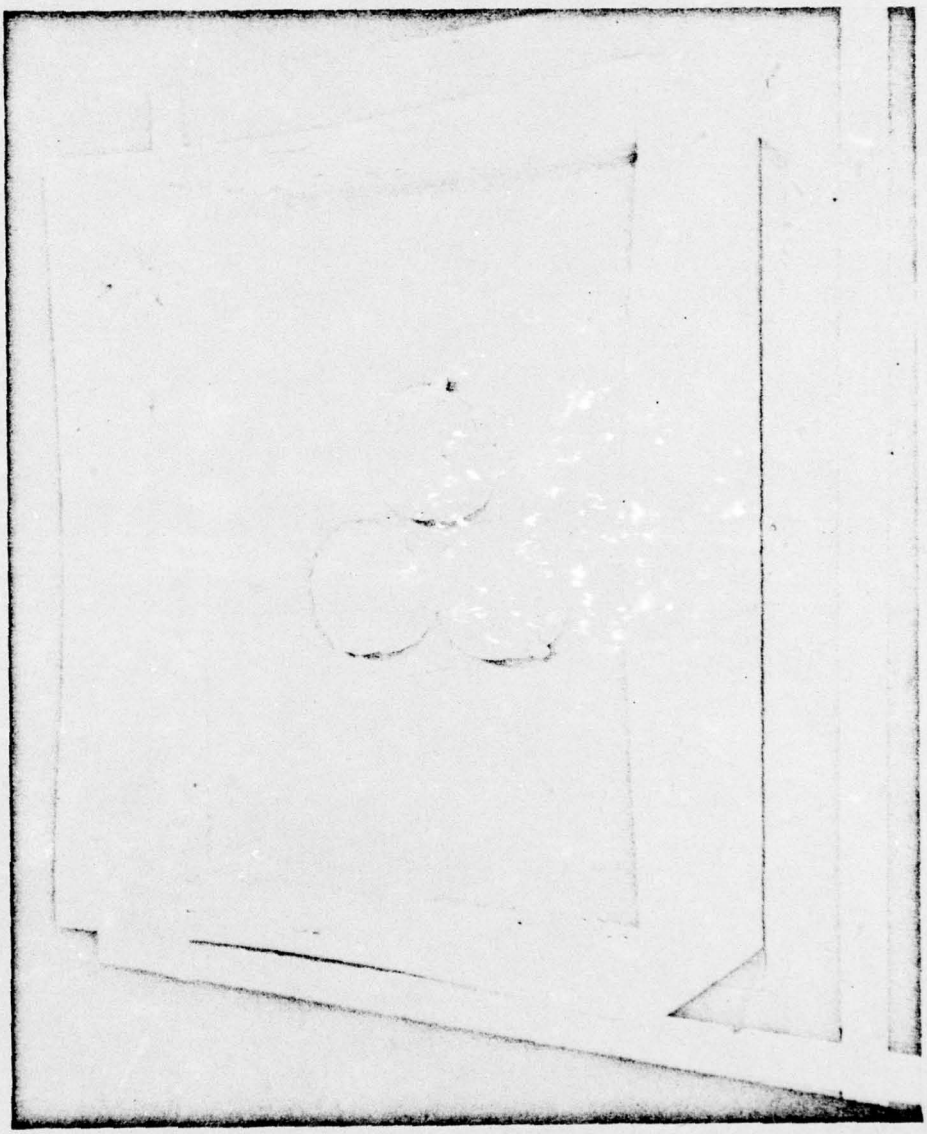


FIGURE 3 FLY CONTAINERS FOR EXPOSURE TO ELF ELECTROMAGNETIC FIELDS

The magnetic field was provided by two large coils. A photograph of the coils is presented in Figure 4. The field variation over the volume of interest was measured to be less than ±2%. The coils are 48 inches with a spacing of 28 inches. Each coil has 236 turns of #14 wire. The required current was 650 ma to produce the 2 gauss magnetic field.

Figure 5 is a block diagram of the driving circuit for the electric field plates and the magnetic field coils. The required phase shift of approximately 90 degrees between the electric and magnetic field components was obtained by deriving the electric field plate voltage from the voltage drop across the magnetic field coil. The resulting phase shift was measured to be 85 degrees.

3. RESULTS

Flies were sexed by observation of the external morphology. Muller-5 males were readily distinguished from the Oregon-R wild type males in terms of eye color and eye shape. Muller-5 females were likewise distinguished from F_1 heterozygous females by marked difference in both eye color and eye shape. Observations were made for only lethal and semi-lethal mutations and no attempts were made to score visible mutations on the X chromosome which would have appeared in the F_2 Oregon-R males. A significant minority of Oregon-R males in a single F_2 culture would indicate a semi-lethal mutation.

Table 1 records the number of successful F_2 cultures in which there were no wild type males found and in which less than half of the males were Oregon-R. A total of 294 X chromosomes were analyzed for the controls with 287 and 298 for the 45 Hz and 75 Hz series, respectively.

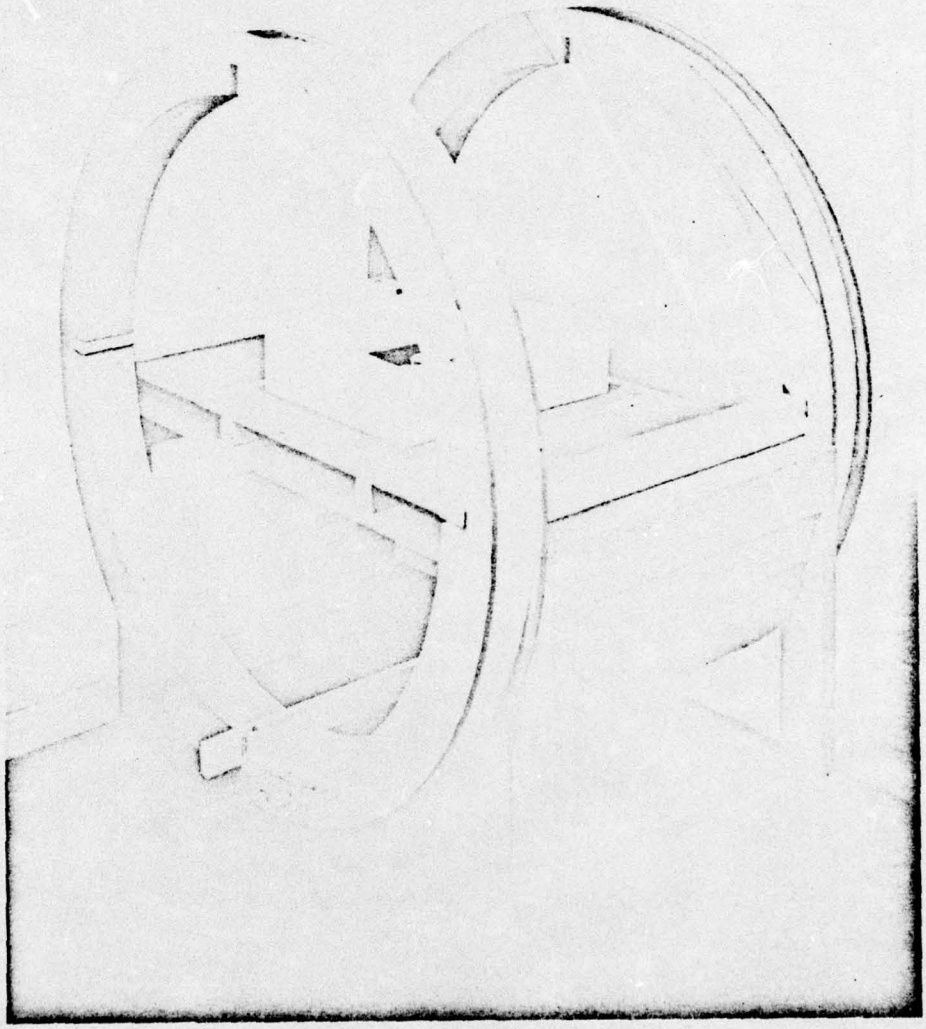


FIGURE 4 MAGNETIC FIELD COILS FOR FRUIT FLY EXPOSURE

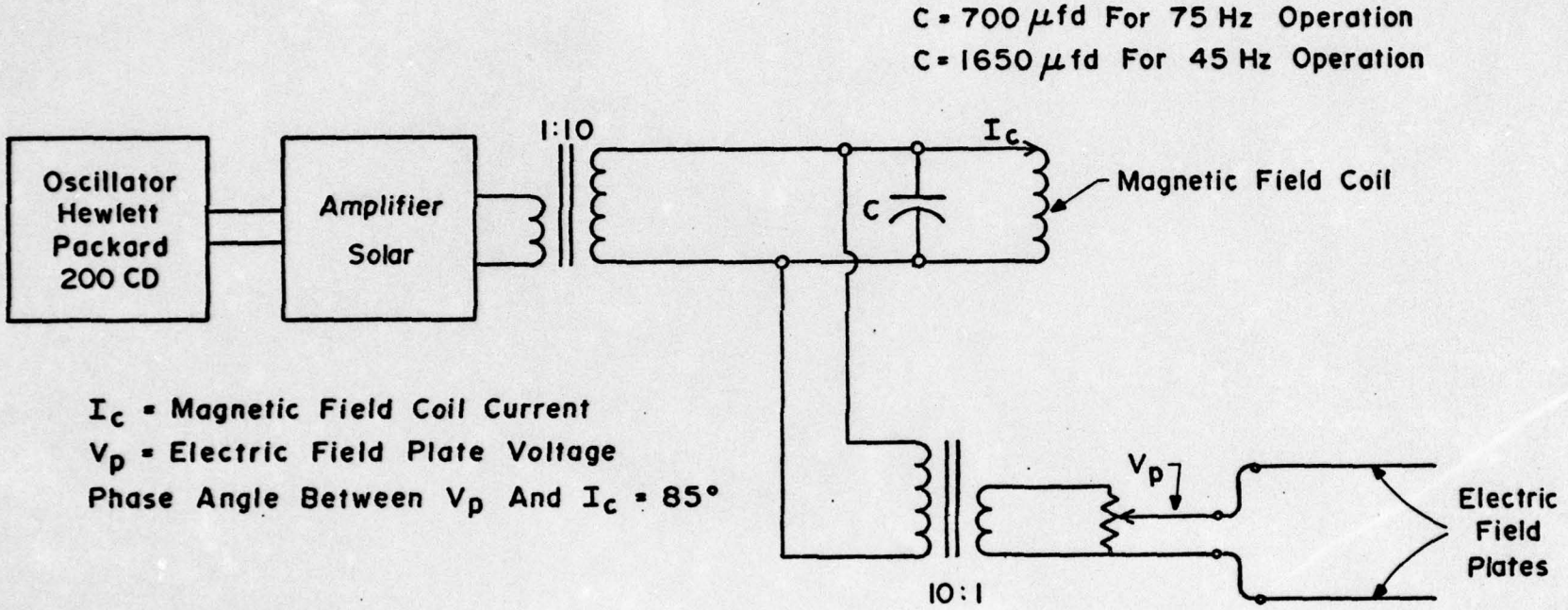


Figure 5 DERIVATION OF E-FIELD PLATE VOLTAGE AND B-FIELD COIL CURRENT

Table 1 Incidence of successful F_2 cultures

(a) lacking Wild-Type (Oregon-R) Males or
(b) with Wild-Type Comprising Fewer than 50% of the Males

	Treatment		
	Control	45 Hz	75 Hz
F_2 cultures analyzed	300	300	300
No. successful	294	277	298
No. without wild-type males	0	0	0
No. with less than 50% wild-type males	41	35	38

No cultures lacking wild type males were found among the control or experimental cultures. Forty-one, thirty-five, and thirty-eight cultures containing less than 50% wild-type males were found among the control, 45 Hz and 75 Hz cultures respectively. However, when these were tested by a Chi-square analysis these were found not to be significant at the 5% level. Cultures of these flies were maintained for two subsequent generations and were never found to show a significant reduction in the number of wild type males and are hence not regarded as representing semi-lethals.

Table 2 presents the total numbers of each sex type obtained in the F_2 along with the percentage of females and males of each type. No significance is noted between the control and the experimental series.

4. DISCUSSION

The fact that no F_2 cultures were found which lacked wild type males would strongly suggest no mutagenic effect of the ELF electromagnetic fields tested. With respect to semi-lethals the same conclusion is indicated.

Table 2 Total Number and Percentage of Each Sex and Type in F_2

	Control		45 Hz		75 Hz	
	N	%	N	%	N	%
Females	37,618	51.30	35,438	51.10	38,025	50.42
Wild-Type males	18,847	25.70	17,329	25.00	19,036	25.25
Muller-5 males	16,866	23.00	16,575	23.90	18,358	24.33

Of the potential semi-lethals observed in the F_2 none showed significant reduction in wild type males in subsequent generations tested.

The 45 Hz series showed fewer successful cultures than either the control or the 75 Hz series. Of the 23 unsuccessful cultures 20 of these were contained within one replicate. The treated males of this replicate could have been deleteriously affected during transportation from IIT Research Institute to Notre Dame.

5. CONCLUSIONS

The sample size in this experiment was of sufficient size to detect a moderate as well as a strong mutagenic effect of the fields tested. Again, the fact that none was found suggests that such weak ELF electromagnetic fields have no mutagenic effect at least within the limits of the sensitivity of the Muller-5 technique. Sufficient dosages were not tested to demonstrate a dose related effect.

6. REFERENCES

1. Coate, W.B., et al., Project Sanguine Biological Effects Test Program Pilot Studies, Final Report, Hazleton Laboratories, Inc., November 1970.

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